Strain typing and laboratory criteria to distinguish *Propionibacterium* species infection from contamination in central nervous system isolates

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ABSTRACT

Background: Laboratory and clinical characteristics that predict infection rather than contamination when *P.* spp. are isolated from the CNS are poorly understood.

Methods: We identified *P.* spp. isolates from the Barnes-Jewish Hospital microbiology lab from 2011-2014. We defined *P.* spp as infectious if treating physicians administered ≥ 7 days of directed antibiotic therapy against *P.* spp.

Results: Of 416 all-site *P.* spp. isolates, 99 were from CNS specimens. Twenty-nine (29%) CNS isolates were considered infections; 23 (79.3%) were *P. acnes* and 6 (20.7%) were *P.* spp. Cultures were from CSF (n=13), wounds involving CNS (n=7), CNS abscess (n=4), CNS aspirates (n=2), CNS tissue, hardware, or cyst (n=1 each). Seventy-one CNS isolates were considered contaminants, 58 (82.9%) were *P. acnes* and 12 (17.1%) were *P.* spp. Patients with 4+ WBCs on Gram stain (n=3) or organisms on Gram stain (n=6) were treated as infections. Compared to patients with contaminated cultures, *P.* spp. infections were more often polymicrobial [9/29 (31%) vs. 2/71 (2.8%) non-infections; p<0.001] or in patients with prior neurosurgical procedure, hardware, or tumor [25 (86%) vs. 16 (22.9%); p<0.001]. Patients with infections had higher CSF nucleated cell counts (median 36 cells/mcl vs. 4 cells/mcl; p=0.002) and protein (median 99.5 mg/dl vs. 41.5 mg/dl; p=0.011), and higher quantification of organisms in culture (median 2+ vs. 1+; p<0.001), Gram stain (median 0 vs. 0; p<0.001), and WBCs on Gram stain (median 1+ vs. 0; p=0.008). CSF glucose was not different between groups (median 66 mg/dl vs. 69 mg/dl; p=0.47). Only 5.8% (n=2) of patients with 0 cells/mcl (n=34) in the CSF were treated; both had CNS hardware. Of the 32 patients with 0 cells/mcl from CSF, 4 had hardware. Phylogroup II was significantly more common in contaminated cultures (p=0.038).

Conclusions: Patients with no CNS instrumentation or pathology, negative Gram stain, and normal CSF parameters are less likely to have a true infection if *P.* spp. are isolated. Phylogroup II appears to be more common in contaminated cultures.

INTRODUCTION

- ***Propionibacterium*** **species are commensal bacteria that are often considered contaminants, but may play an underappreciated role in central nervous system (CNS) infections**
- **Laboratory and clinical characteristics to distinguish infection from contamination have not been established**
- **Previous studies have shown that strain typing can be used to identify pathogenic strain types [1], which may help distinguish pathogenic and non-pathogenic isolates with the potential to reduce unnecessary antimicrobial use**

METHODS

- **Chart review of** ***Propionibacterium*** **spp. isolated from CNS from 2011-2014 at Barnes-Jewish Hospital. Protocol below adapted from McDowell [2].**
- **Isolates were considered infectious if treating physicians administered ≥ 7 days of directed antibiotic therapy against** ***Propionibacterium*** **spp. 4**

P. acnes **confirmation via MALDI-TOF with VITEK MS V2.0**

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DNA extraction using MoBIO Bacteremia DNA kit

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PCR

Primer[a]	Specificity	Target genes	Sequence (5' to 3')	Positions
PArA-1	All *P. acnes*	16S rRNA	AAGCGTGAGTGACGGTAATGGGTA	442–465
PArA-2			CCACCATAACGTGCTGGCAACAGT	1118–1095
PAMp-1	Type IA1/IA2/IC	ATPase	GCGTTGACCAAGTCCGCCGA	451–470
PAMp-2			GCAAATTCGCACCGCGGAGC	944–925
PAMp-3	Type IA2/IB	*sodA*	CGGAACCATCAACAAACTCGAA	168–189
PAMp-4			GAAGAACTCGTCAATCGCAGCA	312–291
PAMp-5	Type IC	Toxin, Fic family	AGGGCGAGGTCCTCTTCTACCAGCG	17–41
PAMp-6			ACCCTCCAACTGCAACTCTCCGCCT	321–297
PAMp-7	Type II	*atpD*	TCCATCTGGCCGAATACCAGG	339–360
PAMp-8			TCTTAACGCCGATCCCTCCAT	689–669
PAMp-9	Type III	*recA*	GCGCCCTCAAGTTCTACTCA	641–660
PAMp-10			CGGATTTGGTGATAATGCCA	865–846

[a] For protein-coding housekeeping genes, the primers relate to positions within the open reading frame. For the 16S rRNA gene, the primers relate to positions within the sequence for *P. acnes* NCTC 737 (GenBank accession no. AB042288). Adapted from [2].

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Visualize 10 µL of PCR product on 2% agarose gel

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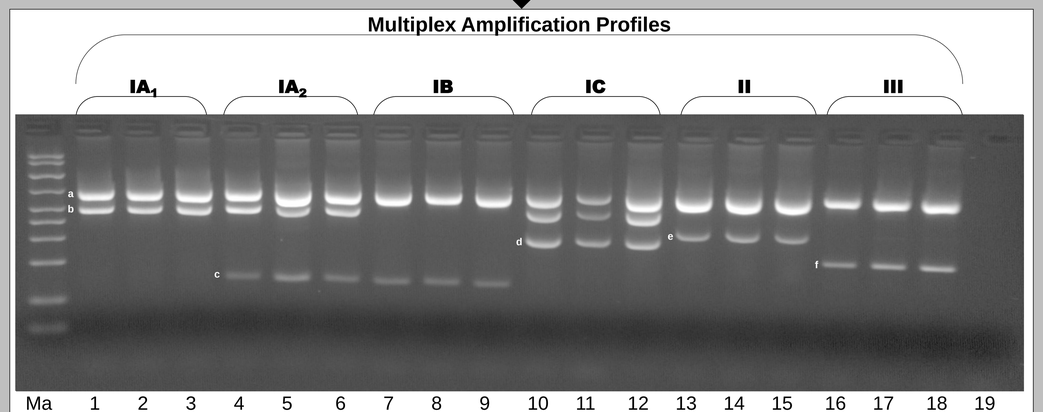
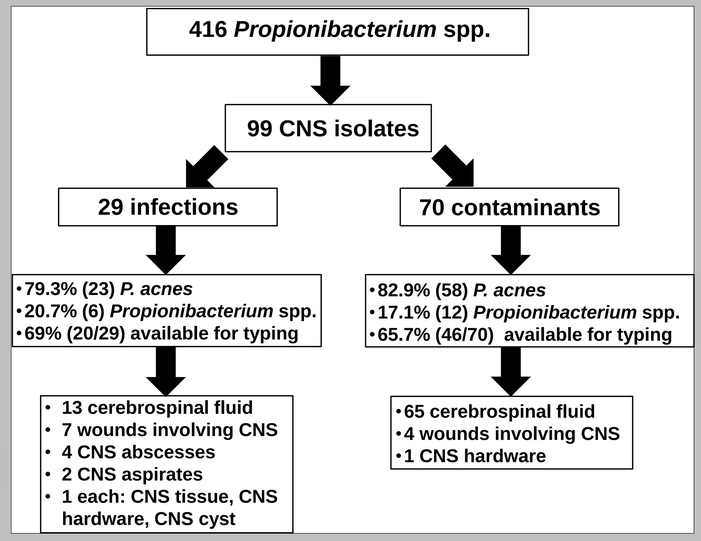


Figure: Multiplex PCR analysis of ***P. acnes*** **strains. Quality controls are lane 1, type IA_1; lane 4, type IA_2; lane 7, type IB; lane 10, type IC; lane 13, type II; lane 16, type III; lane 19, negative control. Lanes 2, 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18 are clinical isolates. Quality control isolates courtesy of McDowell. Gene amplicons (left to right): a, 16S rRNA; b, ATPase; c,** ***soda***; **d, toxin; e,** ***atpD***; **f,** ***recA***.

RESULTS

Culture Flow Chart



Comparison of 99 patients with CNS isolates, by infection status

	Infection	Contaminant	P value
Polymicrobial	31% (9)	2.9% (2)	<0.001
Neurosurgical procedure, hardware, or tumor	86% (25)	22.9% (16)	<0.001
CSF nucleated cell count	36 cells/mcl	4 cells/mcl	0.002
CSF protein	99.5 mg/dl	41.5 mg/dl	0.011
CSF glucose	66 mg/dl	69 mg/dl	0.47
Culture quantification	2+	1+	<0.001
WBCs on Gram stain	1+	0	0.010
Phylogroup IA_1	9	10	NS
Phylogroup IA_2	2	5	NS
Phylogroup IB	6	11	NS
Phylogroup IC	1	1	NS
Phylogroup II	2	16	0.038
Phylogroup III	0	1	NS

Additional Results

- **66 of the 99 CNS isolates were available for strain typing**
- **2 CNS contaminants were classified as non-typeable**
- **All patients with 4+ WBCs on Gram stain or organisms present on Gram stain were classified as infections by treating physicians**
- **Only 5.8% (n=2) of patients with 0 cells/mcl (n=34) in the CSF were treated as infections – both had CNS hardware.**
- **Of 32 patients with 0 cells/mcl in the CSF that were treated as contaminants, 4 had hardware**

CONCLUSIONS

- **Laboratory parameters can be helpful in distinguishing infection from contamination for** ***Propionibacterium*** **spp. isolated from sterile sites**
- ***P. acnes*** **strain typing has the potential to help distinguish infection from contamination in patients with CNS isolates positive for** ***P. acnes***
- **Future research should look to confirm** ***P. acnes*** **phylogroup II as a CNS culture contaminant**

REFERENCES

1. McDowell A, Barnard E, Nagy I, et al. An expanded multilocus sequence typing scheme for *Propionibacterium acnes*: investigation of 'pathogenic', 'commensal' and antibiotic resistant strains. PloS one 2012; 7(7): e41480.
2. Barnard E, Nagy I, Hunyadkurti J, Patrick S, McDowell A. Multiplex Touchdown PCR for Rapid Typing of the Opportunistic Pathogen *Propionibacterium acnes*. J Clin Microbiol 2015; 53(4): 1149-55.